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*Review*

# *TP53* and mutations in human cancer[★✪]

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***TP53* is the most frequently mutated gene in human cancer, with a predominance of missense mutations scattered over 200 codons. In many cancers, specific mutation patterns can be identified, which are shaped by site-specific mutagenesis and by biological selection. In tobacco-related cancers (lung, head and neck), organ-specific patterns are observed, with many mutations compatible with the ones experimentally induced by tobacco carcinogens. In several other cancers, such as squamous cell carcinoma of the oesophagus or hepatocellular carcinoma (HCC), mutation patterns show geographic variations between regions of high and low incidence, suggesting a role for region-specific risk factors. HCC from high-incidence regions showing also a high prevalence of a specific Ser-249 *TP53* mutation is one of the most striking examples of a mutagen fingerprint. All such assessments are useful to generate clues on the mutagenic mechanisms involved in human cancer. Moreover, it has been shown that DNA retrieved from plasma can be successfully used for detection of *TP53* mutations, which gives hope for earlier more accurate detection of human cancers.**

The *TP53* gene is frequently affected by loss of alleles and by point mutations in almost all cancers. *TP53* is located on chromosome 17p13 and encodes a DNA-binding protein with tumour-suppressive properties. The mutations are exceptionally diverse in their position and nature, affecting over 200 codons scattered mainly throughout the central por-

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**Abbreviations:** HBV, hepatitis B virus;HCC, hepatocellular carcinoma; NMSC, non-melanoma skin cancer.

tion of the gene (Hainaut *et al.,* 2000). A database of all published mutations is maintained at the International Agency for Research on Cancer (http://www-p53.iarc.fr/p53database.htm; Olivier *et al.,* 2002). The analysis of *TP53* mutation patterns has shown its usefulness in at least two main areas. First, the position of mutations has helped to better understand the functions of various domains of the p53 protein and their involvement in mediating the suppressive functions that are inactivated in cancer. Second, it has been shown that the patterns of mutations may vary according to the nature of the agents suspected to act as mutagens, allowing the use of *TP53* mutations as a biomarker for the role of carcinogens in human cancer (Hainaut & Hollstein, 2000; Greenblatt *et al.,* 1994). In this brief overview, we will summarise these lessons and we will discuss their implications for the exploitation of *TP53* mutation data, in particular in molecular epidemiological studies. We will also discuss the suitability of free DNA fragments extracted from plasma as a source of material for mutation detection in cancer and pre-cancer patients.

## ROLES OF THE p53 PROTEIN

The main reason why the *TP53* gene is so frequently mutated in cancers (overall in over 50% of invasive cancers) is that the p53 protein plays multiple, coordinated anti-proliferative roles in response to many different types of stress stimuli (Pluquet & Hainaut, 2001; Appella & Anderson, 2000). The conditions that activate p53 include agents that induce DNA damage (UV, gamma or X radiation, bulky carcinogens, alkylating agents, mycotoxins, inhibitors of topoisomerases, etc.) as well as non-DNA damaging conditions such as hypoxia, depletion of ribonucleotides or disruption of cell adhesion. Another category of physiological signals that stimulate p53 results from the activation of growth-promoting signalling cascades. These various signals activate p53 through several pathways, leading to variable speed or level of p53 activation, depending on the nature of the stress, its intensity and the sensitivity of the cell type considered (reviewed in Pluquet & Hainaut, 2001). Thus, there is no uniform "p53 response" and the consequences of p53 induction may vary greatly as a function of various factors (Appella & Hainaut, 2000).

The p53 protein is constitutively expressed in all cell types, but does not accumulate due to its rapid degradation by the proteasome. In response to stimulation, p53 becomes phosphorylated at multiple sites and escapes degradation, as a result of the dissociation from its key regulator, Mdm2, which acts as a ubiquitin ligase to initiate the degradation process. Two main factors can induce the dissociation of p53 from Mdm2. The first one is the phosphorylation of p53 and/or mdm2 on several key residues by stress- or DNA-damage activated kinases (Appella & Anderson, 2000). The second is the sequestration of Mdm2 by the p14(arf) protein. This protein, encoded by the same gene locus as the one encoding the tumour suppressor gene *CDKN2A*, is transcribed in response to growth factors (Sherr, 2000). Therefore, stabilization of p53 can occur in at least two contexts: the response to DNA-damaging stress, and the normal control of cell growth after growth factor stimulation.

Escape from degradation is accompanied by changes in p53 protein conformation that turn it into an active transcription factor. The protein then binds to specific DNA sequences in the regulatory regions of target genes, thus regulating (positively or negatively) their expression. Target genes include regulators of cell cycle, apoptosis, DNA repair and differentiation (Hainaut & Hollstein, 2000). The effect of p53 activation depends upon the time-sequence of regulation of many of these targets. Collectively, these target genes will force the cell to quit DNA replication, either by inducing apoptosis, or by favouring transient or permanent arrest in the cell cycle (Oren & Rotter, 1999).

The p53 protein can be compared to an "emergency brake" that triggers the cell to halt proliferation if conditions are not adequate for correct DNA replication. In normal circumstances, p53 is transiently activated at the G1/S border and participates in the control of the speed and timing of entry into S phase. However, in cells exposed to genotoxic hazards, p53 accumulates to very high levels and it is required to prevent the proliferation of cells that might have acquired new DNA lesions. Loss of TP53 function thus predisposes cells to a rapid accumulation of multiple genetic changes. A principle of this function for cancer progression is provided by the fact that mice without functional *TP53* gene breed and develop (almost) normally but die at an early age from multiple cancers (Donehower *et al.,* 1992). Many of the conditions leading to p53 activation are met during the initial steps of tumour formation (exposure to mutagens, hypoxia, depletion in ribonucleotides or disruption of cell adhesion). Therefore, loss of TP53 function may be critical for cells to bypass a "restriction point" in cancer progression, which would explain why *TP53* mutations are so common in advanced forms of cancer (Guimaraes & Hainaut, 2002).

TP53 belongs to a family of proteins with overlapping functions in growth control, development and differentiation. Two *TP53* homologues, *P63* and *TP73*, encode several splicing variants involved in differentiation and development. Neither of them, however, is a frequent target for missense mutations in human cancers. There is good evidence that p63 plays a central role in the regulation of the differentiation of squamous epithelia, and mice lacking this protein show a dramatic phenotype characterized by the absence of a developed skin and by several cranial and limb malformations. Recent results indicate that *P63* is often overexpressed and sometimes amplified in squamous carcinomas of the head and neck, oesophagus and lung. Thus, this gene may play specific functions in cancer, but very different from those of *TP53* (Levrero *et al.,* 1999).

## *TP53* MUTATION HOTSPOTS — MUTAGENESIS *VERSUS* SELECTION

Most of the mutations in the *TP53* gene occur in the part encoding the DNA-binding domain. In this domain (residues 102 to 296), every residue has been found to be targeted for substitutions in human cancers (with one exception, residue 123) (Olivier *et al.,* 2002). However, some codons are more frequently mutated than others. Mutations at five major "hotspots" account for about 30% of all known mutations. These codons are R175, G245, R248, R249, R273 and R282. The apparent "hypermutability" of these sites is due to two factors. First, these codons encompass CpG sites where cytosines are often methylated, and their spontaneous deamination induces a transition mutation from C to T (Rideout *et al.,* 1990). This type of mutation is frequent in all cancers. Second, these residues play important roles at the surface of contact between the protein and target DNA. Thus, substitution of these residues results in a protein with decreased affinity for DNA, which has lost the capacity to suppress proliferation (Cho *et al.,* 1994). "Hotspot" mutations can thus be explained by an interplay between mutagenesis, which occurs at specific sites, and selection, which gives to cells with deficient TP53 function a selective, proliferative advantage during tumour progression.

There are many mutagens that can damage DNA in specific ways, leaving fingerprints in the genome of cancer cells. However, many of the mutations found in cancers probably arise spontaneously, through endogenous mechanisms. The most common of these mechanisms are polymerase errors during DNA replication or repair and deamination of methylated cytosine in CpG motifs to form thymine. The latter is enhanced by nitric oxide and this type of mutations is common in tumours oc-

curring within a chronic inflammatory context, such as colorectal or stomach cancer (Ambs *et al.,* 1998).

In practice, it is difficult to estimate which mutations arise spontaneously and which ones are due to exogenous carcinogens. Moreover, there is a large degree of overlap between the patterns of mutations from one type of cancer to another. In Fig. 1 we describe factors that influence the formation of mutation patterns as a succession of "filters" allowing the acquisition and persistence of particular mutations (Hainaut & Holstein, 2000). First, the metabolic capacity of exposed cells and tissues determines the extent of DNA-damage induced by a given mutagen. Second, the DNA repair system corrects most alterations and only those which are not repaired are fixed into the genome. Third, biological selection favours cells that have acquired a proliferative advantage as a consequence of a mutation.

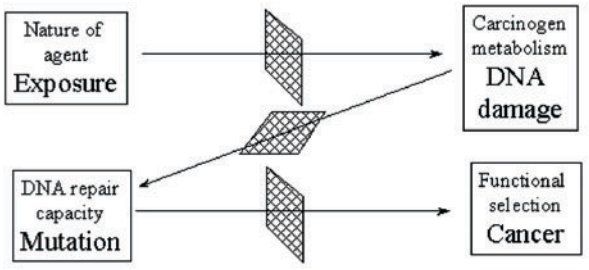


**Figure 1. Interpretation of the formation of mutations as a result of a "succession of biological filters".**

For explanations see text.

## FINGERPRINTS OF ENVIRONMENTAL MUTAGENS

So far, the identification of precise fingerprints left by mutagens in *TP53* has been possible in a number of cancers where there is good, experimental and molecular demonstration that specific carcinogens play an important role. The most significant examples are liver cancer arising in a context of chronic infection by hepatitis viruses and dietary intoxication by aflatoxins, skin cancers (other than melanoma) resulting from exposure to solar radiations, and lung cancers linked with tobacco smoke.

A well-documented mutagen fingerprint is found in non-melanoma skin cancers (NMSC) in relation to sunlight exposure (Daya-Grosjean *et al.,* 1995). The *TP53* mutation spectrum in NMSC shows a high frequency of C to T transitions (56% of all mutations), including tandem CC to TT transitions (6%) that are not found in other tumours. They are due to inefficient repair of a common photoproduct, cyclobutane pyrimidine dimers. In individuals with the inherited syndrome xeroderma pigmentosum, a multi-trait disease associated with hypersensitivity to UV, CC to TT transitions represent about half of all observed mutations, though with important differences depending upon the complementation group of the patient (Giglia *et al.,* 1998). Thus, the CC to TT transitions can be taken as good evidence of the direct DNA-damage generated by exposure to UV.

In lung cancers of smokers, the *TP53* mutation pattern is consistent with mutagenesis by at least some classes of tobacco carcinogens (Pfeifer *et al.,* 2002). Overall, lung cancer differs from cancers unrelated to smoking by a high prevalence of G to T transversions (30% in lung cancer, compared to an average of 9% in non-tobacco related cancers such as brain, breast or colorectal cancers). These transversions are preferentially located on the non-transcribed strand of *TP53* DNA, and often occur at codons 157, 158, 245, 248 and 273. Although data on non-smokers are still limited, it is known that this type of transversion is not frequent in lung cancers of non-smokers (12%) (Pfeifer *et al.,* 2002). Tobacco smoke contains many agents that can potentially induce G to T transversions, par-

ticularly oxidative stress agents, nitrosamines, aromatic amines and polycyclic aromatic hydrocarbons. However, the bases at which the G to T transversions occur in lung cancers of smokers are the same as those where benzo(*a*)pyrene preferentially forms DNA adducts *in vitro* (Smith *et al.,* 2000). It seems that the presence of a methylated cytosine adjacent to a guanine is an important factor for preferential adduct formation. These results clearly indicate a direct mutagenic effect of some major tobacco components in lung cancers of smokers.

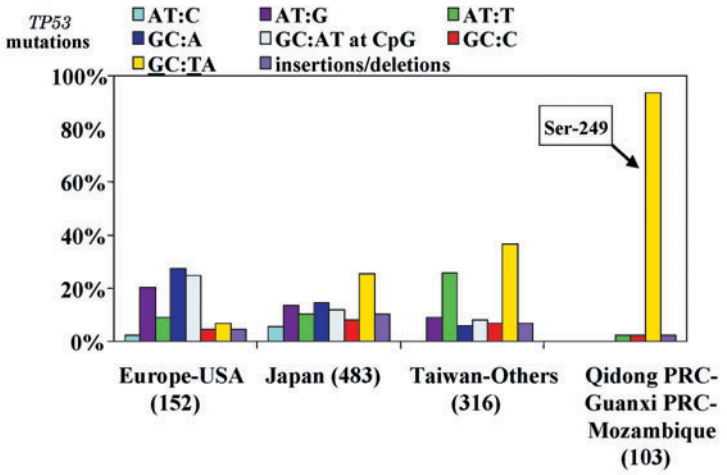


**Figure 2. *TP53* mutation spectra in hepatocellular carcinomas from different regions of the world.**

The numbers in parentheses indicate the number of hepatocellular carcinomas examined. PRC, People's Republic of China (after Montesano *et al.*, 1997, modified based on data from IARC TP53 mutation database).

Hepatocellular carcinoma (HCC) offers one of the most striking example of a mutation "fingerprint" left by a carcinogen in the human genome (Fig. 2). In some areas of the world, such as sub-Saharan Africa and parts of South-East Asia, the co-incidence of aflatoxin $B_1$ ($AFB_1$) exposure and chronic infection by hepatitis B virus (HBV) is responsible for a very high incidence of this kind of tumour in both men and women (40/100 000/year). TP53 is mutated in about 30% of HCCs in low-incidence areas, e.g. Western Europe and the U.S.A., and these mutations are scattered along the DNA-binding domain of the gene, with no particular hotspot. However, in areas of high incidence resulting from alfatoxin $B_1$ exposure and HBV infections, *TP53* is mutated in over 50% of cases, with a high proportion of a single missense mutation at codon 249, AGG to AGT, leading to the substitution of an arginine by a serine (Ser-249) (Montesano *et al.,* 1997). This mutation represents 26% of all *TP53* mutations described to date in HCC and is rather uncommon in other cancers, with no tumour type having more than 2% of Ser-249 mutations. Ser-249 is by far the predominant mutation in areas of high HCC incidence and high aflatoxin exposure, like Mozambique, Senegal, Qidong county in China (Montesano *et al.,* 1997), and The Gambia (our unpublished data). In contrast, the prevalence of Ser-249 is lower in other areas of China as well as in those African and Asian countries (such as Thailand) where average levels of aflatoxin exposure are lower, and it is virtually absent in HCCs from Europe and the U.S.A., where alcohol but not aflatoxin is an important contributor to liver carcinogenesis (Montesano *et al.,* 1997). There is good evidence that metabolites of aflatoxin can induce this mutation *in vitro* as well as in cultured cells. However, this is not the only type of mutations that aflatoxin can generate in *TP53* DNA and the reasons for its almost exclusive presence in tumours of high-incidence areas are not yet elucidated (Denissenko *et al.,* 1998). It is possible that Ser-249 has specific, functional properties conferring a capacity to enhance liver carcinogenesis. Indeed, it is quite remarkable that this mutant is rare in tumours other than HCC.

## PLASMA AS AN ALTERNATIVE SOURCE OF DNA

The major difficulty in *TP53* mutation detection is to make sure that the DNA analysed is representative of the cancer or pre-cancer lesion. These conditions are often met with surgical specimens, in which the pathologist can select representative tumour areas, but it is not always the case with biopsies, in particular those of early lesions. Therefore, it would be very useful to have access to alternative sources of material, such as for example exfoliated tumour cells, that can be obtained with techniques less invasive than biopsy or surgery. Recent studies indicate that plasma DNA may offer such an opportunity.

Free, circulating DNA (CF DNA) is present in the plasma of all individuals. Plasma of healthy subjects contains minute amounts of such DNA (up to a few nanograms per ml), but these levels are increased by 10- to 100- fold in several diseases, including cancer. There is evidence that free plasma DNA often contains mutant DNA from the tumour lesion (Anker *et al.,* 1999). Studies performed in the seventies have demonstrated that in cancer patients, this DNA shows a number of physical characteristics suggesting that it indeed originates from tumour cells. How this cellular DNA reaches the bloodstream is still under discussion. Its release may be a consequence of necrotic and/or apoptotic processes occurring in the tumour. There is also some evidence supporting the hypothesis of active secretion of DNA fragments by normal as well as cancer cells, or release of intact cells into the bloodstream, where their death would liberate their DNA content (Anker *et al.,* 1999).

In recent years, studies using sensitive, PCR-based approach have demonstrated the usefulness of plasma DNA to detect tumour-specific alterations in genes such as *K-RAS* and *TP53,* as well as methylation of *CDKN2A,* microsatellite instabilities, and loss of alleles at specific loci (reviewed in Anker *et al.,* 1999).

In a study on HCC from Western Africa conducted in our laboratory, we have found that the Ser-249 mutation in the *TP53* gene (see above) was detectable in plasma DNA of 38% of HCC patients and that there was an excellent concordance between findings in plasma and in tumour DNA (Fig. 3; Szymańska *et al.*,

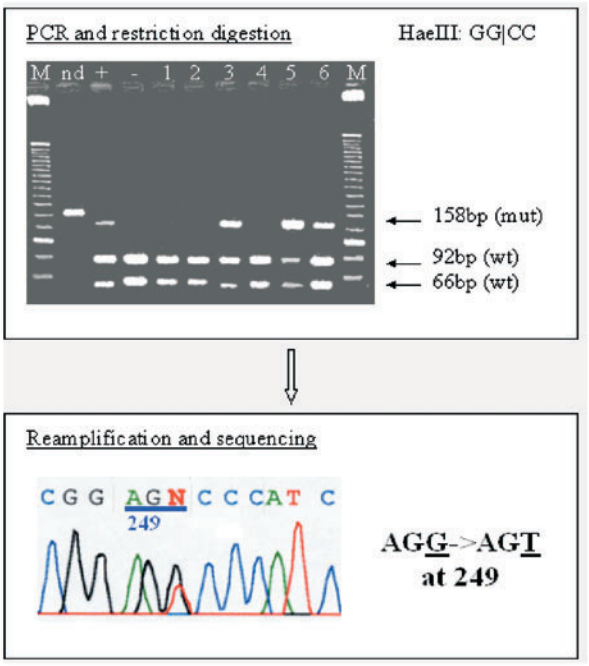


**Figure 3. Detection of Ser-249 mutant DNA in plasma.**

Top: analysis of codon 249 in *TP53* by restriction digestion after amplification of exon 7. Mutation at codon 249 destroys a restriction site, resulting in the presence of a PCR product of 158 bp (see Kirk *et al.*, 2000 for detailed methodology). The first lane (nd) contains a non-digested control. Bottom: sequence analysis of unrestricted DNA, confirming the presence of the mutation.

in preparation). These results suggest that plasma DNA may be a good source of material for *TP53* mutation analysis aimed at early detection of cancer. However, a recent prospective study based on the follow-up of patients with chronic HBV infection and at high risk of liver cancer in the Qidong area (China), does not indicate that Ser-249-mutated DNA is shed in the blood ahead of the occurrence of a

detectable lesion (our unpublished data). In African patients with liver cirrhosis, Ser-249 DNA has been detected in 15 to 20% of cases. Additional studies are needed to assess the prognostic value of these findings.

Another potential interest of plasma DNA lies in the fact that the levels of DNA retrieved show large interindividual differences among cancer patients. Therefore, DNA concentration may be interesting as a prognostic factor and/or even as a potential marker of metastasis in cancer patients.

## CONCLUSIONS AND PERSPECTIVES

With the development of new, sensitive and high-throughput methods for mutation detection, analysis of *TP53* mutations may become an essential aid to the identification of specific cancer risk factors in human populations. In particular, the fact that mutation patterns differ between different geographic areas (for example in HCC) allows one to take advantage of the diversity of human cancers to compare cancer risks in different parts of the world. In clinical terms, the availability of better methods for mutation analysis will help to assess the significance of *TP53* alterations for the diagnosis and prognosis of cancer, in particular if the detection of early mutation can be performed easily on surrogate material. In this respect, further studies on the origin and significance of plasma DNA are certainly needed. Currently, the number of types of *TP53* mutations described in the world literature increases by two to three thousand every year. It is very likely that this trend will continue in the coming years, confirming the status of *TP53* as a central piece of the puzzle in the molecular biology of human cancer.

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